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Determination of major compounds in sweet wines by headspace solid-phase microextraction and gas chromatography

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Abstract

Headspace solid-phase microextraction (HS-SPME) was studied by high resolution gas chromatographic analysis of major compounds (ethyl acetate, methanol, 1-butanol, 2-butanol, 1-propanol, isobutanol, 2-methyl-1-butanol and 3-methyl-1-butanol) in sweet wines. Five different SPME fibres were tested and the influence of different factors such as temperature and time of desorption, extraction time, stirring, sample and vial volume, sugar and ethanol content were studied and optimized using model solutions. The SPME method was validated with the direct injection method. The proposed HS-SPME–GC method is an appropriate technique for the quantitative analysis of the mentioned analytes in real sweet wines.

Keywords: Wine; Headspace analysis; Solid-phase microextraction; Volatile organic compounds

1. Introduction

Wines contain about 800 different volatile compounds belonging to different chemical families such as alcohols, ketones, aldehydes, esters, lactones, etc. [1]. Most of them appear during fermentation processes and their concentrations vary over a wide range. The main technique to analyse these compounds is gas chromatography (GC). The quantification of minor compounds needs a prior concentration step, while major compounds can be analysed by direct injection of wine sample. Among these major compounds are ethyl acetate, methanol, and higher alcohols.

Methanol is originated as a consequence of the hydrolysis of pectines present mainly in grape skin [2]. The methanol is important because of its toxicological properties. The maximum amount legally allowed in wines is 500 mg l^{-1}. Ethyl acetate is the most abundant ester in wines and is produced by the yeast during the alcoholic fermentation and by the acetic bacteria metabolism. High amounts of ethyl acetate can be considered to be a symptom of wine spoil. When the content in ethyl acetate exceeds 200 mg l^{-1}, the organoleptic characteristics typical of acetic acid appear [2]. The process is influenced by several factors such as pH, content and source of nitrogen, fermentation temperature and yeast strain.

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It is known that the content in higher alcohols is a factor conditioning wine quality [3]. Amounts higher than 400–500 mg l^{-1} imply defects in the aroma [4].

For the above reasons and due to the possibility of being analysed by direct injection the enological laboratories have a high demand for analyses of these compounds in wines.

The recommended method of analysis of these compounds in wines and distillates [5] uses GC with packed columns. However capillary columns have also been used to analyse the major compounds wines [6] because they show much better resolution and a shorter analytical time, although their load capacity is lower than that of packed columns.

When sweet wines are analysed by direct injection, due to their high content in sugar and to the high temperature in the injector and in the column, the caramelization of sugars is possible, causing irreversible damage to the column, especially capillary columns. Additionally, the injection of wine samples produces a large amount of particles that can plug column tips causing variation in carrier fluxes and peak shapes. In these cases the solid-phase microextraction (SPME) technique is a better alternative for column protection.

In relation to other extraction techniques used in wine analysis (liquid- and solid-phase extraction, purge and trap, etc.) the SPME offers many advantages because it does not require solvent or any sample treatment. Moreover it is fast, inexpensive, requires low sample volumes and can be easily automated [7].

This technique has successfully been used in wine samples. Thus several authors have studied the aromatic profile of different varietal wines [8–12]. Other studies deal with a single compound such as diacetyl [13], ethyl carbamate [14], *trans*-resveratrol [15], methyl isothiocyanate [16], oxadiaxon [17], 2,4,6-trichloroanisol [18,19], or compounds from the same chemical family such as terpenes [20–23], esters [24], alkylmethoxypyrazines [25,26], sulfurs [27–31], triazoles [32], ethylphenols [33], etc., or compounds from different chemical families [34–37].

In this work, a new method to determine major compounds such as ethyl acetate, methanol, and different higher alcohols (1-butanol, 2-butanol, 2-methyl-1-butanol, 3-methyl-1-butanol, isobutanol, 1-propanol) in sweet wines has been developed and applied to real samples.

2. Experimental

2.1. Material and reagents

The following compounds were studied (CAS number in parentheses): ethyl acetate [141-78-6], 2-methyl-1-butanol [137-32-6], 3-methyl-1-butanol [125-51-3] from Aldrich (Steinheim, Germany, and Milwaukee, WI, USA), methanol [67-56-1], 2-butanol [78-92-2], 1-propanol [71-23-8], isobutanol [78-83-1], 1-butanol [71-36-3] from Fluka (Buchs, Switzerland). 4-Methyl-2-pentanol [108-11-2], 2,3-butanodione [431-03-8] from Fluka (Buchs) and 2-methyl-3-buten-2-ol [115-18-4] from Aldrich, were used as internal standards (I.S.s). Sodium chloride [7647-14-5] was used to control ionic strength (analytical-reagent grade; Merck, Darmstadt, Germany). Absolute ethanol (analytical reagent grade; Merck) [64-17-5] and Milli-Q water (Millipore, Bedford, MA, USA) were used as solvents.

A standard solution containing ethyl acetate (1.32 g l^{-1}) and methanol (4 g l^{-1}) was prepared in 13% ethanol–water and stored at 5 °C. This solution of very volatile compounds was remade weekly. A standard solution of 2-butanol (0.55 g l^{-1}), 1-propanol (0.55 g l^{-1}), isobutanol (0.88 g l^{-1}), 1-butanol (0.66 g l^{-1}), 2-methyl-1-butanol (1.10 g l^{-1}) and 3-methyl-1-butanol (3.3 g l^{-1}) was prepared and stored in the same way. A standard solution of internal standards was prepared in ethanol containing 4-methyl-2-pentanol (1.6 g l^{-1}), 2-methyl-3-buten-2-ol (28.8 g l^{-1}) and 2,3-butanodione (56.0 g l^{-1}). A concentrated synthetic wine solution of 11 g l^{-1} of L-(+)-tartaric acid [87-69-4] (analytical-reagent grade; Merck), 13% ethanol, and sodium hydroxide [1310-73-2] (analytical-reagent grade; Panreac, Barcelona, Spain) to reach pH 3.4 was prepared. In some cases saccharose [57-50-1] (analytical-reagent grade; Panreac) was used to reproduce a standard sweet white wine.

Class A volumetric flasks, Gilson pipetmans regularly verified for precision and accuracy, a precision balance (Sartorius BP 210-S), a pH meter (WTW,

pH 197-S) and a mechanical shaker (Selecta, Rotabit) were used to prepare solutions.

2.2. SPME fibres

The fibres used (Supelco, Bellefonte, PA, USA) were coated with different stationary phases and various film thicknesses: polydimethylsiloxane 100 μm (PDMS-100), polydimethylsiloxane 7 μm (PDMS-7), polydimethylsiloxane–divinylbenzene 65 μm (PDMS–DVB), polyacrylate 85 μm (PA) and Carbowax–divinylbenzene 65 μm (CW–DVB). They were conditioned before use by inserting them into the GC injector under the following conditions: PDMS-100, 250 °C for 1 h; PDMS-7, 320 °C for 3 h; PDMS–DVB, 260 °C for 0.5 h; CW–DVB, 250 °C, for 0.5 h; and PA, 300 °C for 2 h.

2.3. Chromatography

The analyses were carried out on a 3400 GC gas chromatograph equipped with an 8200 Standalone autosampler, a 1077 split/splitless injector and a flame ionization detection (FID) system (Varian, Walnut Creek, CA, USA). The injection was made in the split mode with a 1/20 split ratio, using a liner of 0.75 mm I.D. which improved the GC resolution. The temperature of the detector was 300 °C and it was fed with 30 ml min^{-1} of hydrogen, 300 ml min^{-1} of synthetic air and 30 ml min^{-1} of nitrogen as make-up gas.

The separations were performed using a CP Wax 57 CB Chrompack capillary column (50 m×0.25 mm I.D., 0.20 μm film thickness) with an injector temperature of 250 °C (valid for all the fibres) and an oven temperature programme of 50 °C (4 min), 8 °C min^{-1}, 180 °C (5 min). The carrier gas was helium with a column-head pressure of 20 p.s.i. (1 p.s.i.= 6894.76 Pa).

2.4. Solid-phase microextraction procedure

Optimisation solutions of the extraction process were prepared taking 4 ml of the concentrated synthetic wine solution and 2.3 g of sodium chloride were added to a 16-ml vial, followed by 1 ml of both analyte standard solutions, 50 μl of internal standard solution, ethanol and water to give a 5.5 g l^{-1} solution in tartaric acid, 13% (v/v) ethanol, pH 3.4, and the following standard concentrations: ethyl acetate, 165 mg l^{-1}; methanol, 500 mg l^{-1}; 2-butanol, 68.75 mg l^{-1}; 1-propanol, 68.75 mg l^{-1}; isobutanol, 110 mg l^{-1}; 1-butanol, 82.5 mg l^{-1}; 2-methyl-1-butanol, 137.5 mg l^{-1}; 3-methyl-1-butanol, 412.5 mg l^{-1}; 2,3-butanodione, 350 mg l^{-1}; 2-methyl-3-buten-2-ol, 180 mg l^{-1} and 4-methyl-2-pentanol, 10 mg l^{-1}. The vial was tightly capped with a PTFE-lined cap and then shaken for 10 min at 200 rpm. The fibre was exposed in the headspace for 20 min with solution shaking and then transferred to the injector to be desorbed (250 °C, 2 min).

All studies were made at room temperature (22±1 °C) in triplicate and average values calculated.

2.5. Determination in real samples using direct injection procedure

To validate the SPME procedure, the concentration of real samples of sweet wines from the Canary Islands were determined by direct injection of 3 μl of sample using 4-methyl-2-pentanol as internal standard and a packed Carbowax 1500 column (15% Chromosorb 80–100 mesh, 4 m×1/8 in.) (1 in.=2.54 cm).

3. Results and discussion

Since the final aim of this work is to determine the analytes in sweet wines, which can have a high content (up to 200 g l^{-1}) in sugars, working in direct immersion mode leads to a rapid degradation of the surface of the fibre. To avoid this effect, all the studies were performed in headspace sampling mode.

Fig. 1 shows the chromatograms of a synthetic wine with all the compounds and three real samples of wines where a good separation and resolution among the different peaks can be seen.

Peak identification was accomplished by comparison of the retention times with the standards in the synthetic wine sample.

The optimization of thermal desorption has an important influence on precision, sensitivity, retention time and peak shape [38]. We tested the type of injection (split/splitless), desorption time (0.5–5

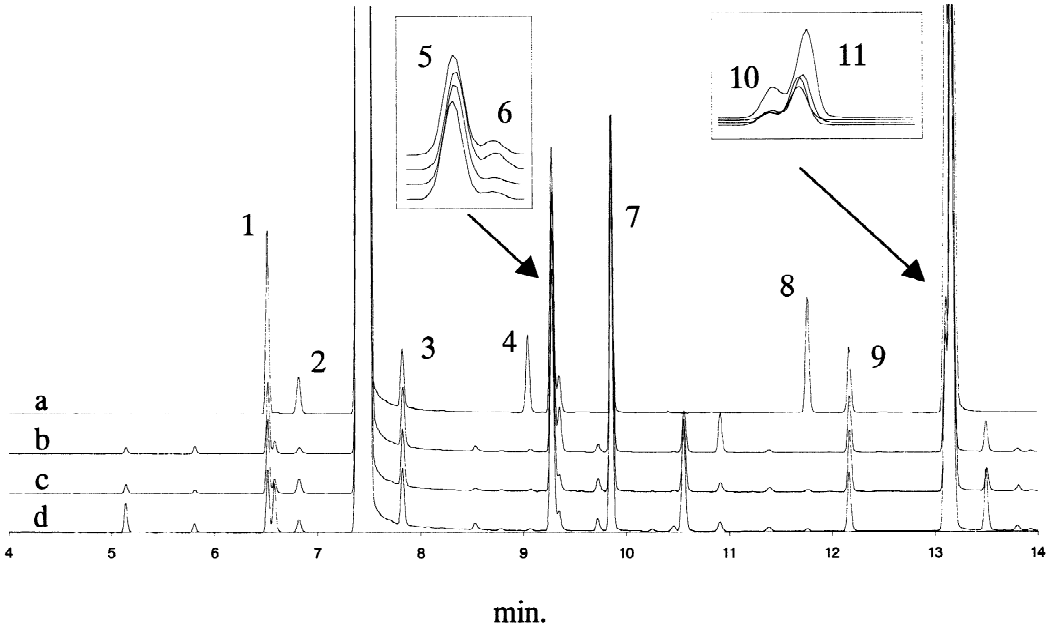

Fig. 1. Chromatogram of a synthetic wine (a) and three sweet wines (b, c, d). 1=Ethyl acetate; 2=methanol; 3=2,3-butanodione; 4=2-butanol; 5=2-methyl-3-buten-2-ol; 6=1-propanol, 7=isobutanol, 8=1-butanol; 9=4-methyl-2-pentanol; 10=2-methyl-1-butanol; 11=3-methyl-1-butanol.

min) and temperature (100–340 °C) for each fibre in the injector. The desorption of the analytes was completed and peak shape corrected using the split mode (1:20), 250 °C as injector temperature and 2 min as desorption time, for all the fibres.

To select the best fibre, they were exposed for 45 min to the optimization solution (Section 2.4) in headspace mode. The results obtained are presented in Fig. 2. As can be observed, with the exception of ethyl acetate, the higher peak areas were obtained with the CW–DVB, hence this fibre was selected for further studies.

In order to optimize the absorption process the factors that influence the extraction equilibria such as

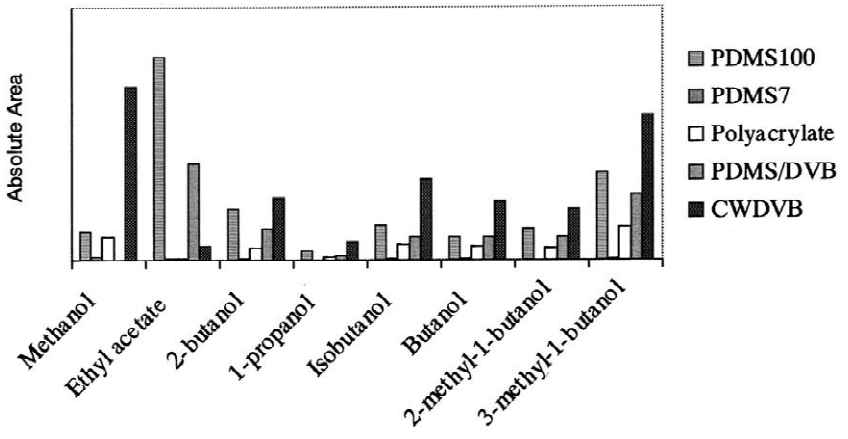

Fig. 2. Extraction profile obtained with different fibres for all the analytes.

extraction time, agitation, sample volume, vial volume, sugar content and ionic strength were considered. Although the optimisation of temperature is not possible in an 8200 SPME autosampler according to several authors [36,39] the absorption of this type of compounds showed a significant decrease with the increase in temperature. Thus working at room temperature (22±1 °C), as in our case, will provide better responses than working at high temperature.

Fig. 3 shows the influence of the extraction time (0–60 min) for every compound using the CW–DVB fibre. The study was performed in 16-ml vials, 13% ethanol, saturated in NaCl, 1:1 phase ratio, with fibre in headspace and stirring. As can be seen, after 5 min the increase in peak areas changed very little for most of the compounds, reaching the highest extraction between 10 and 20 min. Subsequent analyses were therefore performed using a 20-min exposure time.

A study of stirring vs. static sampling was performed and results showed very similar or slightly higher peak areas for all the compounds using stirring mode. Thus, this technique was selected for further studies.

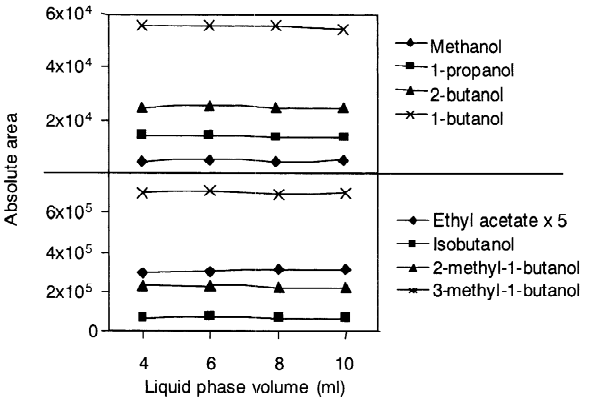

Fig. 4. Variation of peak area as a function of sample volume. Ethyl acetate values appear multiplied by 5.

It is known that phase ratio can affect extraction efficiency [23,38]. Using 16-ml vials we tested 4, 6, 8 and 10 ml of sample volume (phase ratio=3.0; 1.7; 1.0 and 0.6, respectively) at the same concentration of analytes, with stirring and 20-min extraction time. The results obtained showed that the peak areas of the different compounds remain constant over the entire volume range (Fig. 4). This behaviour can be

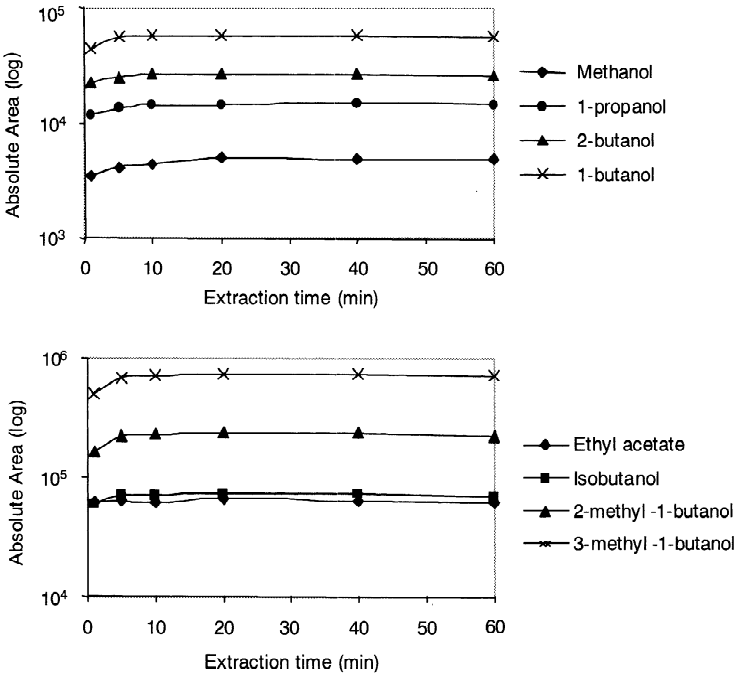

Fig. 3. Extraction curves for the analytes. Absolute peak areas in logarithmic scale.

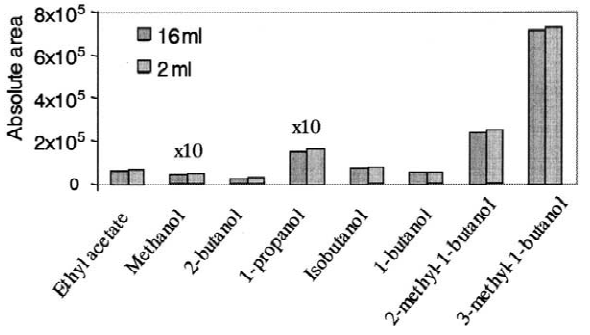

Fig. 5. Comparison of extraction using 2-ml and 16-ml vials. Methanol and 1-propanol values appear multiplied by 10.

explained due to the high concentration of analytes, which readily saturate the vapor phase even for low sample volume.

Two types of vials for automatic SPME, 2 and 16 ml, are commercially available. To test the influence of the vial size we also tested the 2-ml vial, under the same conditions. The study was performed by triplicate with 1.5 phase ratio because the 2-ml vial did not allow sample volume higher than 0.8 without causing partial fibre immersion. The results obtained (Fig. 5) showed that a similar amount of all compounds was extracted in both vials. However the 16-ml vials showed a tendency for a lower RSD than the 2-ml vial, Table 1. Thus, the 16-ml vial was selected for further studies.

The influence of the sodium chloride concentration in the solution (from 0% to saturation) on the extraction was studied. With the exception of methanol, which remained nearly constant, all peak areas increased with the amount of salt (Fig. 6), attaining plateaus when the solution was saturated. Thus, 2.3 g of sodium chloride was added per 16-ml vial.

Table 1
RSD (%) for extraction with 2-ml and 16-ml vials

Compound	16 ml	2 ml
Ethyl acetate	3.56	3.84
Methanol	4.03	5.11
2-Butanol	1.55	2.09
1-Propanol	1.48	2.00
Isobutanol	1.63	2.16
1-Butanol	1.33	1.81
2-Methyl-1-butanol	0.80	1.89
3-Methyl-1-butanol	0.88	1.61

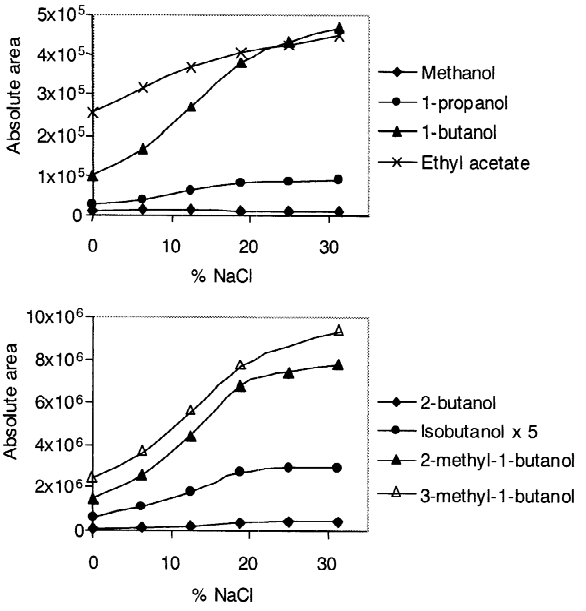

Fig. 6. Variation of extraction performance with NaCl concentration.

To ascertain if sugar can affect the extraction of volatile compounds an extraction study varying the saccharose content (0–200 g l^{-1}) in the synthetic wine was performed. As can be seen in Fig. 7, the extraction of the analytes is not affected by saccharose content. This means that the headspace microextraction technique could be applied to sweet wines without interference of the significant amounts of sugar present in these wines.

After water, ethanol is the second most important component of wine and like other volatile compounds is also extracted in the fibre. Thus it is important to take the ethanol content into account when quantitative analysis is performed [18,24,27,40]. A study of extraction as a function of ethanol content 9–15% (v/v), the range of ethanol for wines, was carried out. Fig. 8 shows that the absolute areas for all analytes and internal standards were independent of the alcoholic concentration.

The calibrated solutions were prepared in 16-ml vials, 13% ethanol, 1:1 phase rate, saturated in NaCl, stirring, fibre in headspace and 20 min extraction time. The concentration ranges were selected according to the concentration of these compounds in wines and three internal standards, 4-methyl-2-pentanol,

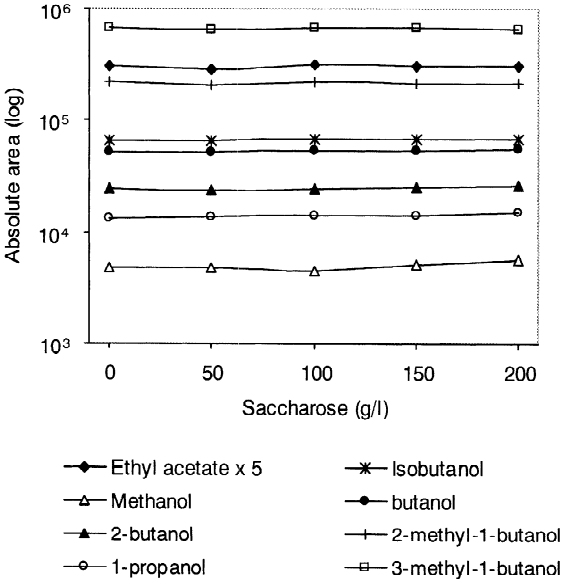

Fig. 7. Extraction profile of analytes with sugar content variation. Ethyl acetate values appear multiplied by 5.

2,3-butanodione and 2-methyl-3-buten-2-ol, were tested.

Sixteen real samples of sweet wines from the Canary Islands were analysed using the optimised SPME procedure and the direct injection method in order to validate the SPME procedure because wine samples contain compounds other than sugars and ethanol that may interfere in the extraction [12,23,41] of the analytes. These real samples were fortified with 4 mg l^{-1} of 1-butanol and 2-butanol because Canary Island wines showed very low concentrations of these compounds. Likewise, this comparative study permitted us to select the best internal standard for each analyte in the SPME procedure.

Table 2
Results of paired *t*-test for selected internal standard and the assigned internal standard for 16 wine samples

Compound	*t* calculated	Internal standard
Ethyl acetate	1.7846	2-Methyl-3-buten-2-ol
Methanol	1.4373	2,3-Butanodione
2-Butanol	0.3859	4-Methyl-2-pentanol
1-Propanol	0.3943	2-Methyl-3-buten-2-ol
Isobutanol	1.7406	4-Methyl-2-pentanol
1-Butanol	1.6781	4-Methyl-2-pentanol
2-Methyl-1-butanol	0.4654	4-Methyl-2-pentanol
3-Methyl-1-butanol	0.6032	4-Methyl-2-pentanol

Critical *t* value=2.26.

To test the concordance between both methods a paired *t*-test of the concentrations obtained was performed using the three internal standards in SPME procedure. Results obtained for each compound and the internal standard selected are presented in Table 2. It can be seen for all the analytes that the calculated *t*-value is lower than critical value

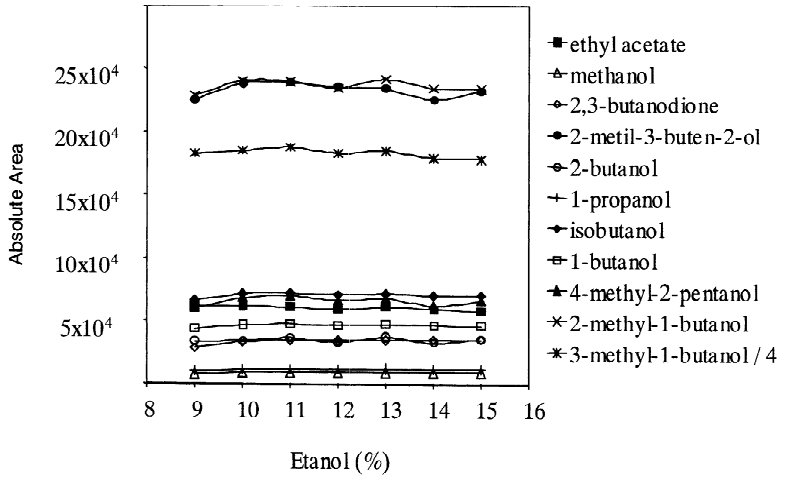

Fig. 8. Variation of peak area for all the analytes and internal standards with ethanol content. 3-Methyl-1-butanol values appear divided by 4.

Table 3
Limits of detection (LODs), range of concentrations, correlation coefficient (r) and relative standard deviations (RSDs) of six replicates

Compound	LOD (mg l^{-1})	Concentration range (mg l^{-1})		r	RSD (%)
		Min.	Max.		
Ethyl acetate	9.6	16.4	171.2	0.9987	5.0
Methanol	10.8	50.3	525.0	0.9998	5.6
2-Butanol	1.9	8.0	80.4	0.9997	3.4
1-Propanol	6.1	6.3	63.2	0.9961	3.5
Isobutanol	2.5	11.4	114.5	0.9998	3.7
1-Butanol	1.5	7.3	73.1	0.9998	4.2
2-Methyl-1-butanol	3.0	14.4	144.0	0.9998	5.5
3-Methyl-1-butanol	4.2	41.3	413.7	0.9999	4.9

of t (2.26), so the null hypothesis is retained, which means that there is no statistically significant difference between both techniques and thus the SPME procedure can be used to determine these analytes in real samples of sweet wines.

The range of concentrations studied, limit of detection, coefficient of regression and repeatability for every compound are presented in Table 3. Limits of detection were determined as three times the noise of six blank injections. The obtained values ranged from 1.5 mg l^{-1} for 1-butanol to 10.8 mg l^{-1} for methanol. A linear regression analysis of relative peak areas referred to the respective internal standard versus the analyte concentration was performed. The application of lack of fit test showed that the F-ratio calculated was not significant for all compounds. The values of the correlation coefficients (r) were higher than 0.996. The repeatability was estimated by the relative standard deviation (RSD) of the area relative to the selected internal standard for six consecutive solutions. All the values obtained were lower than 6% ranging from 3.4% for 2-butanol to 5.6% for methanol.

In the Canary Islands, sweet wines are produced in two different ways, one as naturally sweet wines, with overmatured grapes, without the addition of alcohol or sugar, while others are elaborated as fortified wines, with partial fermentation and subsequent addition of alcohol. There are significant differences in prices between the two types of wines, the naturally sweet wines being considerably more expensive. Hence, the interest in characterising them to avoid possible fraud.

The optimised SPME procedure has been applied to 15 samples of naturally sweet wines and 36 samples of fortified sweet wines. The mean values and standard deviation for each analyte and type of wine are presented in Table 4. None of the wines presented 1-butanol and 2-butanol in detectable amounts. As can be seen, the naturally sweet wines presented a higher content in all the analytes since this type of wines has a longer alcoholic fermentation than fortified wines, which only undergo partial fermentation.

When a principal component analysis (PCA) is applied to the entire set of samples using the determined variables, two main components are obtained with an eigenvalue higher than 1, that explain 84.5% of the total variance of the system. If these two first principal components are plotted, Fig. 9, a clear differentiation is observed between the

Table 4
Mean, standard deviation (SD) and range of concentrations found in different Canary Island sweet wines (mg l^{-1})

Compound	Naturally sweet (n=15)				Fortified (n=36)			
	Mean	SD	Min.	Max.	Mean	SD	Min.	Max.
Ethyl acetate	156.7	33.9	115.8	225.5	65.1	37.2	10.2	138.5
Methanol	103.6	30.3	62.3	158.4	47.1	23.7	0.0	108.5
Propanol	30.1	8.9	20.0	46.2	16.2	7.0	0.0	36.6
Isobutanol	55.6	18.4	30.6	85.7	20.4	11.5	0.0	44.4
2-Methyl-1-butanol	40.2	12.1	19.9	56	20.4	11.7	0.1	46.9
3-Methyl-1-butanol	186.8	57.3	111.4	289.5	110	59.2	0.0	224.5
Amylic alcohols	227.1	68.3	131.3	344.7	130.4	70.5	0.1	270.5
Higher alcohols	312.8	74.3	187.6	421.9	167.1	86.4	0.6	341.0

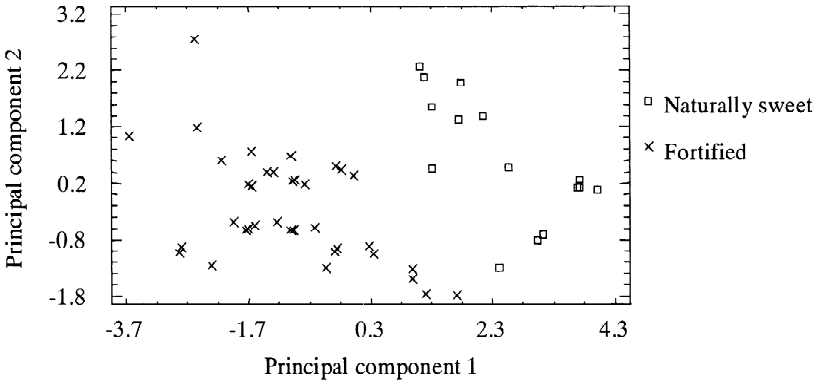

Fig. 9. Scores of the sweet wine samples on axes representing the first two principal components.

samples, in accord with the elaboration procedure employed.

4. Conclusions

A method for the determination of major compounds in sweet wines has been optimised using headspace microextraction combined with high resolution gas chromatography. Five different fibres were tested and the CW–DVB fibre selected. Different parameters that influence the extraction have been optimised and 20 min extraction time, headspace technique, stirring, saturated in sodium chloride and 16-ml vials were selected. The ethanol and sugar content do not influence the extraction which allows this technique to be applied to sweet wine samples. The results obtained in the SPME procedure agreed with those obtained using the direct injection method. The developed SPME method has been applied to samples of commercial sweet wines. Applying PCA to the obtained results established a differentiation of wines according to the type of elaboration process employed.

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References

[1] A. Rapp, in: H.F. Linskens, J.F. Jackson (Eds.), Wine Analysis, Springer-Verlag, Berlin, 1988, p. 29.
[2] L. Usseglio-Tomasset, in: Química Enológica, Mundi-Prensa, Madrid, 1998, p. 111.
[3] A. Bertrand, Conn. Vin 3 (1968) 179.
[4] P. Etievant, in: Volatile Compounds in Food and Beverages, Marcel Dekker, New York, 1991, p. 483.
[5] MAPA, Métodos Oficiales de Análisis, Vol. Tomo II, Ministerio de Agricultura, Pesca y Alimentación, Madrid, 1994.
[6] J.E. Conde, M.A. Rodríguez, J.J. Rodríguez, H. Cabrera, J.P. Pérez, in: III Jornadas Técnicas Vitivinícolas de Canarias, 2000, p. 136.
[7] C.L. Arthur, L. Killiam, K. Buchholz, J. Pawliszyn, J. Berg, Anal. Chem. 64 (1992) 1960.
[8] G.Y. Vas, K. Koteleky, M. Farkas, A. Dobo, K. Vekey, Am. J. Enol. Vitic. 49 (1998) 100.
[9] J. Baptista, T. Tavares, R. Carvalho, Food Res. Int. 34 (2001) 345.
[10] M. Bonino, R. Schellino, C. Rizzi, R. Aigotti, C. Delfini, C. Baiocchi, Food Chem. 80 (2003) 125.
[11] J.C.R. Demyttenaere, C. Dagher, P. Sandra, S. Khalithraka, R. Verhé, N. Kimpe, J Chromatogr. A, in press.
[12] D. De La Calle, M. Reichenbächer, K. Danzar, C. Hurlbeck, C. Bartzsch, K.H. Feller, J. High Resolut. Chromatogr. 20 (1997) 665.
[13] Y. Hayasaka, E. Bartowsky, J. Agric. Food Chem. 47 (1999) 612.
[14] R.S. Whiton, B.W. Zoecklein, Am. J. Enol. Vitic. 53 (2002) 60.
[15] T. Luan, G. Li, Z. Zhang, Anal. Chim. Acta 424 (2000) 19.

[16] N. Gandini, R. Riguzzi, J. Agric. Food Chem. 45 (1997) 3092.
[17] A. Navalón, A. Prieto, L. Araujo, J.L. Vilchez, J. Chromatogr. A 946 (2002) 239.
[18] C. Fisher, U. Fisher, J. Agric. Food Chem. 45 (1997) 1995.
[19] T.J. Evans, C.E. Butzke, S.E. Ebeler, J. Chromatogr. A 786 (1997) 293.
[20] J. García, J. Aleixandre, V.J. Lizama, I. Alvarez, Alimentaria November (1999) 83.
[21] D. De la Calle, S. Magnaghi, M. Reichenbächer, K. Danzar, J. High Resolut. Chromatogr. 19 (1996) 257.
[22] D. De la Calle, M. Reichenbächer, K. Danzer, C. Hurlbeck, C. Bartzsch, K. Feller, Fresenius J. Anal. Chem. 360 (1998) 784.
[23] D. De la Calle, M. Reichenbächer, K. Danzar, J. High Resolut. Chromatogr. 21 (1998) 373.
[24] J.J. Rodríguez, J.E. Conde, M.A. Rodríguez, F. García, J.P. Pérez, J. Chromatogr. A 963 (2002) 213.
[25] C. Sala, M. Mestres, M. Martí, O. Busto, J. Guash, J. Chromatogr. A 880 (2000) 93.
[26] C. Sala, M. Mestres, M. Martí, O. Busto, J. Guash, J. Chromatogr. A 953 (2002) 1.
[27] M. Mestres, O. Busto, J. Guasch, J. Chromatogr. A 808 (1998) 211.
[28] M. Mestres, C. Sala, M.P. Martí, O. Busto, J. Guasch, J. Chromatogr. A 835 (1999) 137.
[29] M. Mestres, M.P. Martí, O. Busto, J. Guasch, J. Chromatogr. A 849 (1999) 293.
[30] M. Mestres, M.P. Martí, O. Busto, J. Guasch, J. Chromatogr. A 881 (2000) 583.
[31] M. Mestres, O. Busto, J. Guash, J. Chromatogr. A 945 (2002) 211.
[32] C.G. Zambonin, A. Cilenti, F. Palmesano, J. Chromatogr. A 967 (2002) 255.
[33] M. Monje, C. Privat, V. Gastine, F. Nepveu, Anal. Chim. Acta 458 (2002) 111.
[34] S. Francioli, M. Guerra, E. López-Tamames, J.M. Guayadol, J. Caixach, Am. J. Enol. Vitic. 50 (1999) 404.
[35] M.A. Pozo-Bayón, E. Pueyo, P.J. Martín-Alvarez, M.C. Polo, J. Chromatogr. A 922 (2001) 267.
[36] E. Marengo, M. Aceto, V. Maurino, J. Chromatogr. A 943 (2002) 123.
[37] R.S. Whiton, B.W. Zoecklein, Am. J. Enol. Vitic. 51 (2000) 379.
[38] J. Pawliszyn, in: Solid Phase Microextraction: Theory and Practice, Wiley–VCH, New York, 1997, p. 117.
[39] S. Rocha, V. Ramalheira, A. Barros, I. Delgadillo, M.A. Coimbra, J. Agric. Food Chem. 49 (2001) 5142.
[40] L. Urruty, M. Montury, J. Agric. Food Chem. 44 (1996) 3871.
[41] H. Guth, J. Agric. Food Chem. 45 (1997) 3022.